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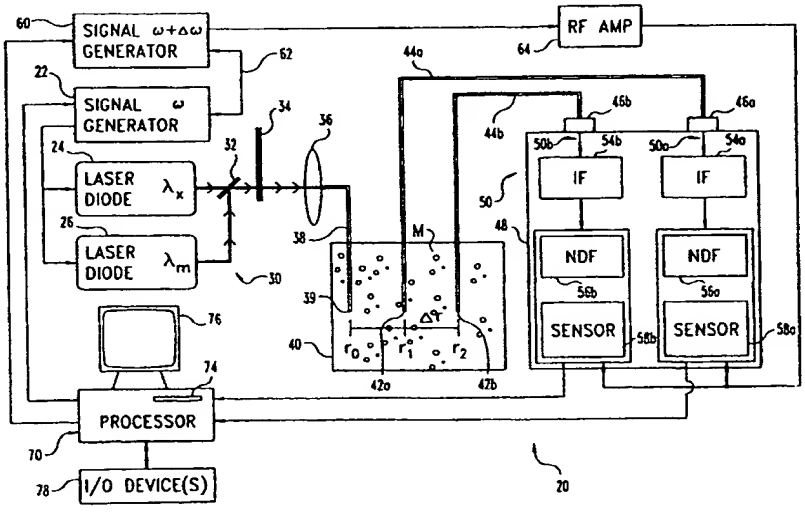

(57) Abstract

A system (20) of the present invention includes light source instrumentation (30) to selectively illuminate a light scattering medium including a luminophore and detection instrumentation (50) to detect multiply scattered light output from the medium in response to illumination by the light source instrumentation (30). A processor (70) is operatively coupled to the detection instrumentation (50) to determine a first optical characterization of the medium from a first multiply scattered light output of a first illumination light wavelength and a second optical characterization of the medium from a second multiply scattered light output of a second illumination light wavelength different than the first illumination light wavelength. The processor (70) is operable to calculate lifetime of the luminophore from the first optical characterization, the second optical characterization, and a multiply scattered emission of the luminophore from the medium in response to excitation.

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CHARACTERIZATION OF LUMINESCENCE IN A SCATTERING MEDIUM

BACKGROUND

The present invention relates to spectroscopic techniques involving luminescence, and more particularly, but not exclusively relates to the determination of lifetime of a luminophore in a light scattering medium.

There has been significant development of fluorescent and phosphorescent dyes or probes with decay kinetics dependent upon the presence or concentration of an analyte or metabolite. Accordingly, lifetime of these dyes can be measured to detect corresponding analyte(s) and/or metabolite(s) concentration. Fluorescent probes in the near-infrared range appear particularly promising for in vivo biomedical diagnostic techniques that involve external, noninvasive measurements or minimally invasive, endoscopic measurements of emitted light.

Unfortunately, quantitative lifetime measurements for such probes are often difficult to obtain in the light scattering environment typically encountered with in vivo diagnostics. Light scattering also hampers other applications of luminophore probes both inside and outside the biomedical field. Consequently, lifetime measurements are usually restricted to dilute, nonscattering solutions. Notably, even equipment used in this manner, such as a curvette to contain the dilute solution, tends to scatter light to some degree introducing an attendant inaccuracy.

Moreover, current lifetime measurement approaches have other limits – especially for fluorophore probes. For example, deconvolution of instrument function often hampers accurate time-domain measurement of lifetimes. In another example, frequency-domain approaches generally require a reference fluorophore with known lifetime characteristics in the environment of interest and at the appropriate excitation and emission wavelengths. Thus, there is a need for further contributions that address these limits and/or other drawbacks confronting this technology.

SUMMARY OF INVENTION

Accordingly, one form of the present invention is a unique technique to evaluate a medium including a luminophore. Other forms include unique systems and methods to measure optical properties of a luminophore in a light scattering medium.

In another form, a light scattering medium includes a luminiphore that is exposed to a number of different wavelengths. Multiply scattered light from exposure to these wavelengths is measured, and one or more optical characteristics of the medium are determined relative to the different wavelengths.

In still another form, an optical characteristic of a luminophore in a light scattering medium is determined. For this form, the medium is illuminated by light at a first wavelength corresponding to an emission wavelength of the luminophore and at a second wavelength corresponding to an excitation wavelength of the luminophore. Multiply scattered light in response to illumination by the different wavelengths is detected to optically characterize the medium and/or luminophore.

A further form of the present invention is a technique to determine the lifetime of a fluorophore. This technique includes both a method and instrumentation directed to fluorescence lifetime measurements. Preferably, this technique does not utilize a reference fluorophore and is not adversely impacted by any light scattering and absorption that might occur in a scattering medium.

Still a further form of the present invention includes a frequency-domain approach to measuring fluorescence lifetime of one or more fluorophores. This approach may include exciting a fluorophore in a light scattering sample with a modulated excitation light and detecting phase shift information. The fluorescence lifetime may be determined from the phase shift information through relationships characterizing light scattering by the sample.

In yet a further form, a light scattering sample containing a fluorophore is exposed to an intensity modulated light at a first wavelength selected to cause the fluorophore to fluoresce at a second wavelength of light. Scattered light at the first wavelength is detected and scattered light at the second wavelength is detected. Optical properties are determined from the detected scattered light to provide fluorescence lifetime based on relationships that characterize photon migration in a light scattering medium. This form may include characterizing the detected scattered light in terms of phase shift in the frequency domain.

In an additional form, a system of the present invention includes an intensity modulated excitation light source configured to deliver an excitation light of a first wavelength to a light scattering substance containing a fluorophore of interest. The fluorophore responds to the excitation light to provide a light emission at a second wavelength. Scattered light is detected at two locations spaced apart from one another. A processor gathers information corresponding to the detected scattered light and processes this information to determine fluorescence lifetime by applying relationships that characterize photon migration of scattered light. For this form, a first detector may be used for detection of light at a first one of the locations that includes a first optical fiber coupled to a first sensor. Also a second detector may be used for detection of light at a second one of the locations that includes a second optical fiber coupled to a second sensor. The first and second detectors may also include corresponding first and second optical filters to selectively detect the first wavelength of light with the first sensor and the second wavelength of light with the second sensor. The coupling arrangement of the first and second fibers to the first and second sensors may be interchanged to obtain comparative measurements for the minimization of equipment inaccuracies.

Further forms, embodiments, objects, features, aspects, advantages, and benefits of the present invention shall become apparent from the detailed description and drawings of the present application.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a schematic view of a system according to one embodiment of the present invention.

Figs. 2A and 2B depict a flow chart illustrating one process that may be performed with the system shown in Fig. 1.

Fig. 3 is a schematic view of a system of another embodiment of the present invention.

Fig. 4 depicts normalized excitation and emission spectra for DTTCI (top) and ICG (bottom) in a comparative format pertinent to experimental examples.

Fig. 5 plots phase shift versus modulation frequency for two different concentrations of DTTCI in a comparative format pertinent to experimental examples.

Fig. 6 plots phase shift versus modulation frequency for two different concentrations of ICG in a comparative format pertinent to experimental examples.

Fig. 7 plots lifetime versus modulation frequency for DTTCI and ICG corresponding to the plots of Figs. 5 and 6.

Fig. 8 plots relative modulation attenuation versus modulation frequency for two different concentrations of DTTCI in a comparative format pertinent to experimental examples.

Fig. 9 plots relative modulation attenuation versus modulation frequency for two different concentrations of ICG in a comparative format pertinent to experimental examples.

Fig. 10 plots lifetime versus modulation frequency for DTTCI and ICG corresponding to the plots of Figs. 8 and 9.

DESCRIPTION OF PREFERRED EMBODIMENTS

For the purpose of promoting an understanding of the principles of the invention, reference will now be made to the embodiments illustrated in the drawings and specific language will be used to describe the same. It will nevertheless be understood that no limitation of the scope of the invention is thereby intended. Any alterations and further modifications in the described embodiments, and any further applications of the principles of the invention as described herein are contemplated as would normally occur to one skilled in the art to which the invention relates.

As used herein, "lifetime" refers to the mean survival time of an activated luminophore or the mean time between the absorption of an excitation photon and emission of a photon. Further, as used herein "multiply scattered light" refers to light that travels at least five (5) times the mean isotropic scattering length $[(1-g)\mu_s]^{-1}$; where g is the mean cosine of angular scatter and μ_s is the scattering coefficient of the medium.

Fig. 1 illustrates evaluation system 20 of one embodiment of the present invention. System 20 includes light source instrumentation 30, container 40, detection instrumentation 50, and processor 70. Light source instrumentation 30 includes modulation signal generator 22 having a range of selectable output Radio Frequencies (RF). Generator 22 drives two monochromatic light sources in the form of laser diodes 24, 26. Laser diodes 24, 26 are intensity modulated at a selected RF frequency (ω) input from generator 22 to provide light at an excitation wavelength (λ_x) and emission wavelength (λ_m), respectively. Instrumentation 30 also includes kinematic mirror 32 that is operable to successively select between the two laser beams at wavelengths λ_x, λ_m output by the respective laser diodes 24, 26. Light from mirror 32 encounters continuously variable neutral density filter wheel 34 of instrumentation 30 to selectively adjust intensity. Lens assembly 36 of instrumentation 30 collects the light output by neutral density filter wheel 34 for input to optical source fiber 38.

Source fiber 38 enters container 40. Container 40 holds a light scattering medium M including a selected amount of a luminophore as a constituent. Source fiber 38 discharges light at site 39 of medium M corresponding to the origin position r_0 (r=0). Detection instrumentation 50 includes optical detector fibers 44a, 44b having respective light input sites 42a, 42b in medium M. Sites 42a, 42b are spaced apart from each other

and correspond to radial distances r_1 and r_2 relative to r_0; where this spacing is represented by Δr ($\Delta r = r_2 - r_1$).

Interchangeable connectors 46a, 46b couple fibers 44a,44b to detector 48 of detection instrumentation 50. Detector 48 includes two optic channels 50a, 50b coupled by connectors 46a, 46b to receive light from detector fibers 44a, 44b, respectively. Each channel 50a, 50b has a corresponding interchangeable/removable interference filter (IF) 54a, 54b; adjustable neutral density filter (NDF) 56a, 56b; and light sensor 58a, 58b.

Light sensors 58a, 58b are coupled to processor 70 to provide one or more output signals corresponding to light detected from medium M. Sensors 58a, 58b are arranged in a standard heterodyne configuration and may be of any form such as Photomultiplier Tubes (PMTs), photodiodes, or image intensified Charge Coupled Devices (CCDs) to name just a few. For the heterodyne configuration, RF signal generator 60 is phase locked to generator 22 at a slightly different frequency $\omega + \Delta\omega$ as represented by coupling 62; where $\Delta\omega$ is the frequency difference. The output of generator 60 is amplified by RF amplifier 64 and mixed at sensors 58a, 58b to provide a corresponding differential output signal from which information corresponding to phase and modulation magnitude of the detected light can be determined with processor 70. Accordingly, processor 70 is also operatively coupled to generators 22, 60.

Processor 70 includes a port for insertion and removal of a portable memory device such as an electromagnetically or optically encoded disk, cartridge, or tape. In addition to being coupled to light source instrumentation 30 and detection instrumentation 50, processor 70 is also operatively coupled to visual display 76 and one or more Input/Output (I/O) devices 78, including for example a keyboard, mouse, light pen, acoustic loudspeakers, microphone, and/or printer just to name a few. Processor 70 may be comprised of one or more components configured as a single unit, or when of a multi-component form, processor 70 may have one or more components remotely located relative to the others, or otherwise have its components distributed throughout system 20. Processor 70 may be programmable, a state logic machine or other type of dedicated hardware, or a hybrid combination of programmable and dedicated hardware. One or more components of processor 70 may be of the electronic variety including digital circuitry, analog circuitry, or both. As an addition or alternative to electronic circuitry, processor 70 may include one or more optical elements.

Processor 70 includes an integrated and/or remote storage capability in the form of one or more types of memory. By way of nonlimiting example, this memory may include one or more of the solid-state, magnetic, and/or optical memory types. Such memory types may include Random Access Memory (RAM), Sequential Accessible Memory (SAM) (such as the First-In, First-Out (FIFO) variety, or the Last-In, First-In LIFO variety), Programmable Read Only Memory (PROM), Electrically Programmable Read Only Memory (EPROM), flash memory or Electrically Erasable Programmable Read Only Memory (EEPROM); an optical disc memory (such as a CD ROM); a magnetically encoded hard disc, floppy disc, tape, or cartridge; another variety of storage device as would occur to those skilled in the art, or a combination of any of these types. Furthermore, the memory may be volatile, nonvolatile, or a hybrid combination of volatile and nonvolatile varieties. Also, memory may be permanently installed, in a portable form that may be readily removed and reinstalled, or a combination of these types.

In one embodiment including electronic circuitry, processor 70 is of a standard personal computer configuration with a common solid-state digital integrated processing unit operatively coupled to solid-state memory. For this personal computer embodiment, appropriate interfaces are installed to facilitate control of generators 22, 60 and receipt of data from detector 48. The memory of this embodiment contains programming to be executed by the processing unit, and is arranged for reading and writing of data in accordance with one or more routines executed by processor 70. Besides memory, processor 70 may include any oscillators, control clocks, interfaces, signal compensators/conditioners, filters, limiters, Analog-to-Digital (A/D) converters, Digital-to-Analog (D/A) converters, communication ports, or other types of circuits as would occur to those skilled in the art to implement the present invention.

Processor 70 is configured to execute one or more routines to perform selected calculations with data received from detector 48 for lifetime evaluation process 120. Referring additionally to the flow chart of Figs. 2A and 2B, evaluation process 120 is illustrated. Evaluation process 120 utilizes Frequency Domain Photon Migration (FDPM) techniques to extract the characteristic optical properties of medium M. These properties are obtained for photons of two wavelengths: the wavelength used to optically excite a selected luminophore and the wavelength of the luminescence resulting from this excitation. Lifetime measurements are determined from these wavelength-dependent

characterizations of the medium and comparative luminescence measurements, as described hereinafter.

In stage 122 of process 120, a luminiphore probe with known excitation wavelength λ_x and emission wavelength λ_m is selected for evaluation and placed in light scattering medium M. This light scattering medium M may include, for example, living biologic tissue for which metabolites/analytes are being interrogated in terms of lifetime of the selected luminophore probe. In other examples, the light scattering medium M may be a cell culture, flow cytometry stream, a chemical reaction medium or other light scattering environment as would occur to those skilled in the art. In one alternative, endogenous luminophores in medium M are interrogated without introduction of an exogenous probe in stage 122.

In stage 130, intensity modulated light at frequency ω with the excitation wavelength λ_x of the designated luminophore is provided by light source instrumentation 30 to source site 39 of medium M. Accordingly, light from laser diode 24 is provided from light source instrumentation 30 through fiber 38 to site 39. The illuminating light is subsequently scattered and/or absorbed by medium M. As multiply scattered light sourced from site 39 reaches sites 42a, 42b; it can be sensed with detection instrumentation 50 and quantitized in terms of frequency domain parameters of relative phase shift and/or modulation magnitude. Filters of detector 48 are removed and/or adjusted to facilitate detection of the excitation light wavelength with sensors 58a, 58b for this stage.

Photon transport in a light scattering medium M may be modeled as a diffusive process. In the frequency domain, the photon density $U(\mathbf{r},\omega)$ in a homogeneous medium at a vector position $\mathbf{r}$ can be related to optical properties of the medium by the diffusion equation (1) as follows:

$$-cD\nabla^2 U(\mathbf{r},\omega) + (c\mu_a + i\omega)U(\mathbf{r},\omega) = q(\mathbf{r},\omega); \qquad (1)$$

where: $D = [3(\mu'_s + \mu_a)]^{-1}$ is the diffusion coefficient of the medium, c is the speed of light in the medium, $q(\mathbf{r},\omega)$ describes properties of the light source, and ω is the modulation angular frequency of the light source (generator 22). The output signal from detection instrumentation 50, $V(\mathbf{r},\omega)$, depends on the complex responsivity $R(\lambda,\omega)$ expressed as: $V(\mathbf{r},\omega) = R(\lambda,\omega)U(\mathbf{r},\omega)$. The magnitude of $R(\lambda,\omega)$ represents gain and conversion

efficiency of the photon transport and the angle represents the phase delay of the modulated light.

For the diffusion equation model, the absorption coefficient μ_a and the isoptropic scattering coefficient μ'_s characterize pertinent light scattering properties of the medium at a given light wavelength. The isotropic scattering coefficient is related to the scattering coefficient μ_s by: $\mu'_s = (1-g)\mu_s$; where g is the mean cosine of angular scatter. As used herein, the subscripts "x" and "m" are used to designate various optical parameters specific to the excitation and emission wavelengths, respectively. It has been found that lifetime measurements in a multiply scattering medium may be performed by accounting for the optical characteristics of the medium at two light wavelengths commonly associated with the luminophore: (a) the excitation wavelength λ_x and (b) the emission wavelength λ_m. This characterization can be in terms of the absorption and isotropic scattering coefficients for each of the two wavelengths: (a) μ_{ax}, μ'_{sx} for λ_x and (b) μ_{am}, μ'_{sm} for λ_m.

Accordingly, in stage 130 medium M is characterized at λ_x beginning with operation 132. In operation 132, medium M is exposed to modulated light of wavelength λ_x at site 39 as sourced from laser diode 24 of light source instrumentation 30. This source light can be modeled as an isotropic point source of the form: $q_x(\mathbf{r},\omega) = P_x(\omega)\delta(\mathbf{r})$; where $P_x(\omega)$ is a complex number that represents the source magnitude and phase and $\delta(\mathbf{r})$ is the Dirac delta function. Furthermore, by modeling the light behavior in terms of the diffusion equation with infinite boundary conditions, the solution to the diffusion equation takes the form of spherical photon density waves described by the following expression (2):

$$U_x(r,\omega) = \frac{P_x(\omega)}{4\pi c D_x r} e^{-k_x(\omega) r} \qquad (2)$$

where: $U_x(\mathbf{r},\omega)$ is the frequency domain excitation photon density in the medium M and the complex wave vector $k_x(\omega)$ is given by expression (3) as follows:

$$k_x^2(\omega) = \frac{\mu_{ax}}{D_x}\left(1 - i\frac{\omega}{c\mu_{ax}}\right). \quad (3)$$

On substitution of expression (3) into expression (2), the excitation photon density can be modeled as follows in expression (4):

$$U_x(r,\omega) = \frac{P_x(\omega)}{4\pi c D_x r} e^{-\beta_x(\omega)r} \left(\cos(\gamma_x(\omega)r) + i\sin(\gamma_x(\omega)r)\right) \quad (4)$$

where:

$$\alpha_x(\omega) = \left(\sqrt{\left(\frac{\mu_{ax}}{D_x}\right)^2 + \left(\frac{\omega}{c D_x}\right)^2}\right)^{1/2}$$

$$\beta_x(\omega) = \alpha_x(\omega)\cos\left(\frac{1}{2}\tan^{-1}\left(\frac{\omega}{c\mu_{ax}}\right)\right)$$

$$\gamma_x(\omega) = \alpha_x(\omega)\sin\left(\frac{1}{2}\tan^{-1}\left(\frac{\omega}{c\mu_{ax}}\right)\right).$$

The photon density is related to the observed modulation phase $\theta(\mathbf{r},\omega)$ by expression (5a) as follows:

$$\tan\theta(r,\omega) = \frac{\mathrm{Im}U(r,\omega)}{\mathrm{Re}U(r,\omega)}. \quad (5a)$$

The modulation $M(\mathbf{r},\omega)$ of the photon density waves a distance r away from and normalized to unity at the source (r = 0) is related to photon density by expression (5b) as follows:

$$M(r,\omega) = \sqrt{\frac{\mathcal{R}e^2\,U(r,\omega) + \mathcal{I}m^2\,U(r,\omega)}{\mathcal{R}e^2\,U(r,0) + \mathcal{I}m^2\,U(r,0)}}. \quad (5b)$$

For injection of source light at the excitation wavelength into the medium M in operation 132, the substitution of expression (4) into equations (5a) and (5b) results in expressions

(6a) and (6b), respectively, as follows:

$$\theta_x(\mathbf{r},\omega) = \gamma_x(\omega)\mathbf{r} \qquad (6a)$$

$$M_x(r,\omega) = \frac{P_x(\omega)}{P_x(0)}\, e^{r[\alpha_x(0) - \beta_x(\omega)]}. \qquad (6b)$$

The relative modulation phase and magnitude are observed with detection instrumentation 30 at the two radial distances r_1 and r_2; where $\Delta r = r_2 - r_1$, $r_2 > r_1$. The resultant phase difference is expressed as: $\Delta\theta_x(\Delta r,\omega) = \gamma_x(\omega)\Delta r$; and the resultant modulation magnitude may be as follows in expression (7):

$$M_x(\Delta r,\omega) = \frac{M_x(r_2,\omega)}{M_x(r_1,\omega)} = e^{\Delta r[\alpha_x(0) - \beta_x(\omega)]}. \qquad (7)$$

where the actual modulation information observed with detection instrumentation 50 is as follows:

$$m_x(r,\omega) = M_x(r,\omega)\, m_{sx}\, m_{dx}(r,\omega) \qquad (8)$$

and is related to $M_x(\mathbf{r},\omega)$ by the modulation of source m_{sx} and the modulation response $m_{dx}(\mathbf{r},\omega)$ of detection instrumentation 50. The ratio of the observed modulation signals is provided by expression (9) as follows:

$$m_x(\Delta r,\omega) = \frac{m_x(r_2,\omega)}{m_x(r_1,\omega)} = \frac{m_{dx}(r_2,\omega)}{m_{dx}(r_1,\omega)}\, e^{\Delta r[\alpha_x(0) - \beta_x(\omega)]} \qquad (9)$$

This ratio is generally independent of source modulation.

In operation 132, data is collected and stored in processor 70 corresponding to detected multiply scattered light output from medium M. It should be appreciated that only one of relative phase and modulation attenuation information needs to be observed to provide the desired characterization of the medium M at the excitation wavelength. In order to calculate both the absorption and isotropic scattering coefficients μ_{ax}, μ'_{sx} with expression (4) from relative phase or magnitude observations, a regression or other iterative estimation can be employed. To facilitate this calculation, operation 132 detects multiply scattered light outputs for a number of different RF modulation frequencies.

While relative measures of phase or modulation attenuation are indicative of photon-migration through medium M, differences in the response function of channels 50a and 50b of detector 48 may result in inaccuracy that reaches an undesirable level for some applications. It has been found that instrumentation response function effects may be reduced by switching from the illustrated (first) configuration with sites 42a, 42b respectively coupled to channels 50a, 50b to a second configuration with sites 42a, 42b coupled to channels 50b, 50a; and repeating the phase and/or modulation magnitude measurements of operation 132 in operation 134 with the second configuration. The measurements of operation 134 are conducted with the same relative spacing between sites 39, 42a, and 42b. Connectors 46a, 46b provide a convenient way to manually perform this reconfiguration. In an alternative embodiment, an optical multiplexer may be utilized to automatically accomplish this reconfiguration under the control of processor 70.

In an example based on relative phase measurements, the effective response function of the detection instrumentation is designated θ_{instr}. Letting $\Delta\theta_x(r_2r_1) = \theta_x(r_2) - \theta_x(r_1) + \theta_{instr}$ represent measurements during operation 132 and $\Delta\theta_x(r_1r_2) = \theta_x(r_1) - \theta_x(r_2) + \theta_{instr}$ represent measurements during operation 134, the instrument effects can be removed by taking one half of the difference between these two relative measurements. The desired phase difference, $\Delta\theta_x$, is then expressed as: $\Delta\theta_x = 0.5[\Delta\theta_x(r_2r_1) - \Delta\theta_x(r_1r_2)]$.

Measurements gathered with detection instrumentation 50 in operations 132, 134 of stage 130 for a desired number of different modulation frequencies are recorded with processor 70. Stage 140 is next encountered to obtain an optical characterization of medium M at the emission wavelength λ_m in terms of absorption and isotropic scattering coefficients μ_{am}, μ'_{sm}. In operation 142, emission wavelength light is provided to source site 39 with laser diode 26 of light source instrumentation 30, instead of excitation

wavelength light from laser diode 24. Filtering of detector 48 is removed/adjusted to facilitate detection of multiply scattered light output from medium M at the emission wavelength λ_m and measurement of relative modulation phase or magnitude over a selected range of modulation frequencies in the manner described for stage 130. The emission photon density is characterized in stage 140 in accordance with expressions (2)-(9) substituting the emission wavelength for the excitation wavelength (and correspondingly substituting subscript "x" with "m" in these expressions). In operation 144, the measurements are repeated with coupling of site 42a being switched to sensor 58b of channel 50b and coupling of site 42b being switched to sensor 58a of channel 50a. The measurements of operations 142, 144 are recorded with processor 70 to determine absorption and isotropic scattering coefficients μ_{am}, μ'_{sm}.

In stage 150, luminescence data is gathered. Excitation wavelength light is provided to site 39 of medium M from laser diode 24 of light source instrumentation 30 in operation 152 of stage 150 and frequency domain measurements are gathered. For stage 150, detection instrumentation 50 is configured with filtering of channel 50a adjusted/removed to detect the excitation wavelength with sensor 58a and filtering of channel 50b adjusted/removed to detect the emission wavelength with sensor 58b. For a fluorescent type of luminophore, fluorescence photon density may be modeled in accordance with expression (10) as follows (where the subscript "f" denotes fluorescence optical parameters):

$$U_f(r,\omega) = \frac{\phi\,\mu_{af}\,P(\omega)}{4\pi c D_x D_m r}\left(\frac{e^{-k_x(\omega)r} - e^{-k_m(\omega)r}}{k_m^2(\omega) - k_x^2(\omega)}\right)\left(\frac{1 + i\,\omega\tau}{1 + (\omega\tau)^2}\right). \qquad (10)$$

The quantum efficiency of the fluorophore is denoted by ϕ and μ_{af} describes absorption of excitation light due to fluorescence. Fluorescence decay is assumed to be of the monoexponential type with lifetime τ; however, the principles of the present invention can be applied to multiexponential decays using techniques known to those skilled in the art. Using expression (3), the fluorescence photon density of expression (10) can be written as presented in the following expression (11):

$$U_f(r,\omega) = \frac{\Phi\mu_{af}P(\omega)}{4\pi cD_xD_m[1+(\omega\tau)^2]r}\{[\psi(r,\omega)-\kappa(r,\omega)\omega\tau] + i[\kappa(r,\omega)+\psi(r,\omega)\omega\tau]\}, \quad (11)$$

where:

$$\psi(r,\omega) = \frac{\delta(r,\omega)\xi + \zeta(r,\omega)\rho(\omega)}{\xi^2+[\rho(\omega)]^2},$$

$$\kappa(r,\omega) = \frac{\zeta(r,\omega)\xi - \delta(r,\omega)\rho(\omega)}{\xi^2+[\rho(\omega)]^2},$$

$$\delta(r,\omega) = \exp[-\beta_x(\omega)r]\cos[\gamma_x(\omega)r] - \exp[-\beta_m(\omega)r]\cos[\gamma_m(\omega)r],$$

$$\zeta(r,\omega) = \exp[-\beta_x(\omega)r]\sin[\gamma_x(\omega)r] - \exp[-\beta_m(\omega)r]\sin[\gamma_m(\omega)r],$$

$$\xi = \frac{\mu_{am}}{D_m} - \frac{\mu_{ax}}{D_x},$$

$$\rho(\omega) = \frac{\omega}{c}\left(\frac{1}{D_x} - \frac{1}{D_m}\right).$$

The fluorescence photon density is related to the observed fluorescence modulation phase $\theta_f(r,\omega)$ by expression (5a). Substituting expression (11) into expression (5a), expression (12) results as follows:

$$\tan\theta_f(r,\omega) = \frac{\kappa(r,\omega)+\psi(r,\omega)\omega\tau}{\psi(r,\omega)-\kappa(r,\omega)\omega\tau}, \quad (12)$$

and the fluorescence decay lifetime can be written per expression (13) as follows:

$$\tau = \frac{1}{\omega}\frac{\tan\theta_f(r,\omega)-\eta(r,\omega)}{\eta(r,\omega)\tan\theta_f(r,\omega)+1}, \quad (13)$$

where $\eta(r,\omega) = \kappa(r,\omega)/\psi(r,\omega)$ and determinations of $\psi(r,\omega)$ and $\kappa(r,\omega)$ are based on measured phase shifts $\Delta\theta_x(\Delta r,\omega)$ and $\Delta\theta_f(\Delta r,\omega)$.

Alternatively, the fluorescence photon density is related to fluorescence modulation $M_f(r,\omega)$ by expression (5b). Substituting expression (11) into expression (5a) and noting that $\kappa(r,0) = 0$ yields expressions (14) and (15) as follows:

$$M_f(r,\omega) = \frac{P(\omega)}{P(0)}\sqrt{\frac{\epsilon(r_2,\omega)}{(1+(\omega\tau)^2)}} \quad (14)$$

where:

$$\epsilon(r_2,\omega) = \frac{\psi^2(r_2,\omega) + \kappa^2(r_2,\omega)}{\psi^2(r_2,0)} \qquad (15)$$

is based upon the optical coefficients of the sample. Comparable expressions can be derived for other types of luminescence and different boundary conditions.

After measurements of relative modulation phase or magnitude are made in operation 152, the measurements are repeated in operation 154 with sites 42a, 42b being switched relative to channels 50a, 50b as described in connection with operation 134 and 144. As part of the reconfiguration for stage 154, the filtering of channels 50a, 50b is also adjusted to facilitate detection of the excitation wavelength with sensor 58b and the emission wavelength with sensor 58a. Measurements of operations 152, 154 are stored with processor 70.

In calculation stage 160 (Fig. 2B) of evaluation process 120, processor 70 performs calculations with the data collected during stages 130, 140, 150. In operation 162, processor 70 performs regression analysis of measurements from excitation characterization stage 130 to determine the absorption and isotropic scattering coefficients μ_{ax}, μ'_{sx} at the excitation wavelength. In operation 164, processor 70 performs regression analysis of measurements from emission characterization stage 140 to determine the absorption and isotropic scattering coefficients μ_{am}, μ'_{sm} at the emission wavelength.

In operation 166, processor 70 calculates lifetime based on the measurements obtained from luminescence characterization stage 150 and the coefficients obtained from operations 162, 164. For calculations of lifetime based on relative phase measurements in stage 150, the measured phase shift $\Delta\theta_f(\Delta r,\omega) = \theta_f(r_2,\omega) - \theta_x(r_1,\omega)$ is adjusted by the addition of phase change $\theta_{xc}(r_1,\omega)$ associated with the excitation propagation from site 39 to site 42a at r_1. This phase value $\theta_{xc}(r_1,\omega)$ can be calculated from expression (6a) and the optical characteristics determined in operation 162 from measurements of excitation wavelength characterization stage 130. The resultant modulation phase $\theta_f(r_2,\omega)$ =

$\Delta\theta_f(\Delta r,\omega) + \theta_{xc}(r_1,\omega)$ is the phase at the source ($r = 0$) and can be used in expression (13) to determine lifetime τ.

For calculation of lifetime based on modulation attenuation, the modulation magnitude at radial distance r_2 is referenced relative to the excitation modulation magnitude at radial distance r_1. The ratio of expression (14) to expression (6b) yields expression (16) and (17) as follows:

$$M_f(\Delta r,\omega) = \frac{M_f(r_2,\omega)}{M_x(r_1,\omega)} = \frac{1}{e^{r_1[\alpha_x(0)-\beta_x(\omega)]}} \sqrt{\frac{\epsilon(r_2,\omega)}{(1+(\omega\tau)^2)}} \qquad (16)$$

from lifetime τ is derived:

$$\tau = \frac{1}{\omega}\sqrt{\frac{\epsilon(r_2,\omega)}{M_f^2(\Delta r,\omega)\ e^{2r_1[\alpha_x(0)-\beta_x(\omega)]}} - 1} \qquad (17)$$

The modulation magnitude detected with detection instrumentation 50 is given by expression (18) as follows:

$$m_f(r_2,\omega) = M_f(r_2,\omega)\ m_{sx}\ m_{dm}(r_2,\omega) \qquad (18)$$

which is related to modulation $M_f(\mathbf{r},\omega)$ by modulation m_{sx} of the source at the excitation wavelength, and the modulation $m_{dm}(r_2,\omega)$ of the detection instrumentation 50 at the emission wavelength. The ratio of expression (18) and the excitation modulation of expression (8) is provided by expression (19) as follows:

$$m_f(\Delta r,\omega) = \frac{m_f(r_2,\omega)}{m_x(r_1,\omega} = \frac{M_f(r_2,\omega)}{M_x(r_1,\omega} \frac{m_{dm}(r_2,\omega)}{m_{dx}(r_1,\omega} \qquad (19)$$

From the ratio of expression (19), the following expression (20):

$$M_f(\Delta r,\omega) = m_f(\Delta r,\omega)\ \frac{m_{dx}(r_1,\omega)}{m_{dm}(r_2,\omega}} \qquad (20)$$

is found to depend upon the ratio of the detection instrumentation 50 response functions at excitation and emission wavelengths. This information may be expressed as a single ratio of modulations of the sources; where given the optical coefficients of the medium M from operations 162 and 164, excitation $M_{xc}(\mathbf{r},\omega)$ and emission $M_{mc}(\mathbf{r},\omega)$ can be determined from expression (7). The product of source modulation and detection instrument response function can be determined from expression (8) at the excitation:

$$m_{sx}\ m_{dx}(r_1,\omega) = \frac{m_x(r_1)}{M_{xc}(r_1,\omega)} \qquad (21)$$

and emission:

$$m_{sm}\ m_{dm}(r_2,\omega) = \frac{m_m(r_2)}{M_{mc}(r_2,\omega)} \qquad (22)$$

wavelengths. The resulting ratio of detection instrument response functions is given by expression (23) as follows:

$$\frac{m_{dx}(r_1,\omega)}{m_{dm}(r_2,\omega)} = \frac{m_{sm}}{m_{sx}}\ \frac{m_x(r_1,\omega)}{m_m(r_2,\omega)}\ \frac{M_{mc}(r_2,\omega)}{M_{xc}(r_1,\omega)} \qquad (23)$$

which depends upon a comparison between measured and calculated modulation information and a constant ratio of modulations of the sources at emission and excitation wavelengths. Varying the source modulation ratio, the lifetime measured at multiple modulation frequencies can be regressed to obtain a unique source modulation ratio associated with a vanishing slope, i.e. minimized χ^2 of the lifetime distribution, and to obtain the resulting lifetime τ. After lifetime is determined in stage 160 from relative phase or modulation measurements, it is output in stage 170, concluding process 120.

It should be understood that only one possible sequence of the measurement stages 130, 140, 150 is illustrated. Indeed, the various measurements may be performed in many other sequences with respect to selected light wavelengths, modulation frequencies, and coupling configuration of sites 42a, 42b relative to channels 50a, 50b. Also, the calculations of stage 160 may be performed any time by processor 70 in relation to the measurement stages 130, 140, 150 to the extent measurement data has been provided to processor 70.

In other embodiments, the arrangement of system 20 may differ. For example, light source instrumentation may include a source of excitation and/or emission wavelength light such as a different type of laser, lamp, or other device as would occur to those skilled in the art. In another example, detection instrumentation 50 may include an optical multiplexer controlled by processor 70 to provide for the switching of optical couplings relative to detector 48. In one alternative embodiment, the measurements may be based on two spaced apart light source sites 39 in medium M with just one detection site 42a or 42b and corresponding sensor 58a or 58b. In one form of this arrangement, light source instrumentation may be configured with another set of the components described for instrumentation 30 to provide two light source channels; while detection instrumentation 50 could be modified to include only a single channel 50a or 50b. The lifetime calculations may be readily adapted to this dual source using techniques known to those skilled in the art. Also, instrument function that might lead to calculation discrepancies relative to the two source sites can be addressed by switching the equipment supplying light to each source; thereby providing the first and second measurement configurations described in connection with stages 130, 140, 150.

Fig. 3 depicts medical diagnostic system 220 of another embodiment of the present invention. System 220 includes diagnostic instrument 225 to evaluate a patient's medical condition based on lifetime readings of an endogenous, exogenous, or immobilized exogenous fluorophore in a patient's tissue 280. Instrument 225 includes light source apparatus 230, detection apparatus 250, and processor 270 that are operationally configured like light source instrumentation 30, detection instrumentation 50, and processor 70, respectively, but are packaged in a manner convenient for in vivo interrogation of tissue 280.

The fluorophore in tissue 280 is typically an exogenous probe introduced into tissue 280 that has a known excitation and emission wavelength to which the optics of

instrument 225 are matched. Such exogenous probes may be immobilized in a subcutaneous implant, by oral ingestion, or any other means that would occur to those skilled in the art. Alternatively or additionally, the present application may be applied to monitor endogenous fluorophores for which excitation/emission wavelengths are known.

Light source apparatus 230 includes one or more sources to provide light to tissue 280 at excitation and emission wavelengths for the selected fluorophore in accordance with evaluation process 120. The source light is provided to site 239 of tissue 280 as represented by arrow IL. The source light is multiply scattered by tissue 280 and received by detection apparatus at sites 242a, 242b as represented by arrows MS1, MS2. Instrument 225 is configured to maintain a generally constant relative spacing between sites 239, 242a, and 242b, corresponding to r_0, r_1, and r_2, respectively. Processor 270 may be configured to control selection of the appropriate source light wavelength and modulation frequencies for light source apparatus 230 and the corresponding filter configuration for detection apparatus 250 to automatically perform the stages and operations of process 120 with instrument 225.

For external interrogations, instrument 225 may include a moveable hand-held wand or optical probe coupled to a base unit; where the wand is arranged for placement proximate to surface 282 of tissue 280. In this case, surface 282 may be the patient's skin, with instrument 225 being arranged to make percutaneous measurements. In other instances, instrument 225 may include an endoscope coupled to a base unit that is arranged to perform interrogations through a body lumen, cavity, or small surgical incision. For these instances, surface 282 can be the boundary of the body lumen or an organ selected for interrogation, to name just a few examples. Depending on the configuration of instrument 225, the calculations performed in stage 160 may be adjusted to account for finite boundary conditions to improve performance. In another embodiment, instrument 225 is arranged to place sites 239, 242a, 242b in the tissue, more closely approximating infinite boundary conditions. Furthermore, in alternative embodiments, instrument 225 may be completely contained in a portable device that is battery powered.

Yet another embodiment includes interrogating a light scattering medium including an amount of a selected luminophore with light at an emission wavelength of the luminophore and sensing multiply scattered light at the emission light wavelength in response to this interrogation to provide a first optical characterization of the medium. The medium is also exposed to light at an excitation wavelength of the luminophore and a

multiply scattered luminescence at the emission wavelength is detected in response to this exposure. A value corresponding to lifetime of the luminophore is determined from the first optical characterization and the luminescence.

A further embodiment includes exposing a light scattering medium including an amount of a luminophore to a number of different light wavelengths selected relative to the luminophore. A plurality of optical characteristics of the medium are established by sensing multiply scattered light at each of the different wavelengths. A value corresponding to lifetime of the luminophore is determined from the optical characteristics and a multiply scattered emission at a first one of the light wavelengths caused by exposure of the medium to a second one of the light wavelengths.

Still a further embodiment provides for an evaluation of a light scattering medium including an amount of a luminophore with a light interrogation system. This system includes a first device optically coupled to a first site of the medium and a second device optically coupled to a second site of the medium that is spaced apart from the first site. Also included are subjecting the medium to a first light wavelength selected relative to the luminophore, optically coupling the first device to the second site and the second device to the first site, and exposing the medium to the first light wavelength after coupling. A value corresponding to lifetime of the luminophore is determined from a first light output and a second light output.

Another embodiment includes means for illuminating a light scattering medium including a luminophore; means for characterizing light scattering behavior of the medium for an excitation wavelength of the luminophore and an emission wavelength of the luminophore; and means for determining lifetime of the luminophore from the characterizing means and a multiply scattered light emission from the medium at the emission wavelength in response to illumination by light at the excitation wavelength.

Also, because luminophores may have different activated states, they may have multiple lifetimes corresponding to these states. While the examples described herein are directed to the lifetime of luminophores that are excited to a single activated state to preserve clarity, in other embodiments the principles of the present invention can be applied to luminophores with multiple activated states and lifetimes using techniques known to those skilled in the art. Such multiple lifetime probes are also within the scope of the present invention.

EXPERIMENTAL EXAMPLES

The present invention will be further described with reference to the following specific examples. These experiments and results are intended to be illustrative of the present invention and should not be considered limiting or restrictive with regard to the scope of the present invention. Further, any theory, mechanism of operation, proof, or finding stated herein is meant to further enhance understanding of the present invention and is not intended to make the present invention in any way dependent upon such theory, mechanism of operation, proof, or finding.

Several experiments were conducted with a test setup comparable to system 20. FDPM measurements according to process 120 were conducted in a light scattering tissue-simulating phantom form of medium M. For these experiments the lifetimes of different micromolar concentrations of two luminophores of the fluorescent type where evaluated: (1) 3,3'-Diethylthiatricarbocyanine Iodide (DTTCI) and (2) Indocyanine Green (ICG or IR-125) (both from AR-COS Organics, Fair Lawn, N.J.). Container 40 for the phantom was a cylindrical acrylic vessel 11.3 cm in diameter and 15 cm in height. The phantom was prepared as an aqueous (DUIF water; Fisher Scientific, Fair Lawn, N.J.) intralipid (20%; Pharmacia & Upjohn Company, Clayton, N.C.) solution to mimic tissue-like scattering properties. A DTTCI stock solution was prepared with ethanol; and ICG was dissolved directly in water. Appropriate quantities of stock solutions were suspended in 1.0% Intralipid to yield the final fluorophore concentrations in the phantoms as listed in Table 1 that follows:

Dye	Conc. [μM]	λ_x [nm]	μ_{ax} [1/cm]	μ'_{sx} [1/cm]	λ_m [nm]	μ_{am} [1/cm]	μ'_{sm} [1/cm]	τ [ns]
DTTCI	0.5	749	0.054(8)	9.2(12)	828	0.032(3)	10.2(8)	1.34(3)
	1.0	749	0.07(2)	6.8(15)	828	0.031(3)	8.4(7)	1.34(4)
ICG	0.0625	778	0.039(8)	9.5(13)	828	0.033(4)	8.0(8)	0.54(3)
	0.125	778	0.05(1)	7.9(20)	828	0.051(9)	9.2(15)	0.56(4)

Additional samples were prepared in water for spectral analysis. Figure 4 shows the concentrations of the analyzed dyes and presents the relevant excitation and emission spectra measured with a spectrofluorometer (Fluorolog-2; SPEX Industries, Inc., Edison, N.J.).

The lifetime of DTTCI dissolved in ethanol was previously reported as 1.33 ± 0.02 ns at 790-nm excitation and 820-nm emission wavelengths. The lifetime of ICG was reported to be 0.57 ± 0.02 ns at 780-nm excitation and 830-nm emission wavelengths measured without scattering by use of time-domain techniques as well as by conventional frequency-domain fluorimetry referenced against the known lifetime of a well-characterized fluorophore.

For the experimental examples, excitation wavelengths were provided by laser diodes of the 56 DFS series; Melles Griot, Boulder, Colorado with power/wavelengths of 3 mW at 749nm for ICG excitation and 25 mW at 778nm for DTTCI excitation. A 30 mW 830 nm laser diode from Melles Griot was used for emission wavelengths. Kinematic mirror 32 was a model 9891 from New Focus, Santa Clara and neutral density filter wheel 34 was from Newport of Irvine, California. Source fiber 38 was a 1000-μm-core source fiber (3M FT SILICA/0.39-NA TECS multimode fiber type). Also, fibers 44a, 44b were provided by two additional 1000-μm-core fibers of identical length placed in medium M at equal depth and parallel to source fiber 38. Detector fibers 44a, 44b were positioned within the sample at distances r_1 = 1.0 cm and r_2 = 2.5 cm away from source fiber 38. The radial separation distance (Δr = 1.5cm) of the two detection fibers remained unchanged during all measurements. The light sensed by the fibers was delivered to sensors 58a, 58b in the form of gain-modulated photomultiplier tube (PMT) detectors (Model H6573; Hammamatsu, Bridgewater, N.J.). The detector fibers 44a, 44b were terminated with connectors 46a, 46b in the form of SMA fiber-optic connectors that could be interchanged conveniently to perform the reconfigurations of stages 130, 140, 150.

Detector 48 included neutral-density filters 56a, 56b from CVI Laser Corporation, Albuquerque, N.M. and interference filters 54a, 54b of a narrow-bandpass form (10nm FWHM; CVI Laser Corporation). Also included were lens assemblies that focused the collected light onto the PMTs. The gain settings of the PMTs remained unchanged during all measurements. The three neutral-density filters in the setup aided in maintaining constant dc levels of the detected PMT signals. The filters 34, 54a, 54b were used to adapt the setup to different output power levels of the two source laser diodes 24, 26 and to

facilitate acquisition of light intensity signals over a large dynamic range. The PMTs were gain modulated at the modulation frequency of the laser diodes plus an offset frequency of 100 Hz ($\Delta\omega$). The heterodyned PMT signals were then digitized, Fourier transformed, and analyzed for phase shift and amplitude attenuation by use of LabVIEW software (National Instruments Corporation, Austin, Tex.) executed by a personal computer form of processor 70.

The experimental results illustrated in Figs. 5-7 are based on relative phase measurements in stages 130, 140, 150. To obtain these results the stages were performed in a different order than illustrated in the flow chart of Figs. 2A and 2B. Namely, Stage 150 was performed first, followed by stage 130, and then stage 140. The measurement protocol for the lifetime determination comprises the three stages 130, 140, 150 for each concentration; where each stage includes measurements for two different configurations over a range of modulation frequencies from about 60 to about 140 MegaHertz (MHz). The kinematic mirror placed in the experimental setup expedited execution of the three stages of each measurement. Optical parameters of the sample at excitation and emission wavelengths were obtained from two-parameter least-squares fits of expression (5) to the excitation and the emission data, respectively. These optical coefficients together with the fluorescence phase shift then permitted the deduction of the lifetime of the fluorescent dye from expression (13).

The top graph (a) of Fig. 5 provides experimentally determined plots of phase shift versus modulation frequency for a 0.5μM concentration of DTTCI with the excitation characterization measurements of stage 130, the emission characterization measurements of stage 140, and the fluorescence characterization measurements of stage 150 shown in ascending order. The bottom graph (b) of Fig. 5 provides plots of the same type and in the same order as top graph (a) for a 1.0μM concentration of DTTCI. The top graph (a) and the bottom graph (b) of Fig. 6 provide excitation, emission, and fluorescence characterization plots in the same order as the graphs of Fig. 5 for 0.0625μM and 0.125μM concentrations of ICG, respectively. Fig. 7 plots the lifetime calculation results of stage 160 for the two concentrations for DTTCI (hollow symbols) and ICG (solid symbols) as based on the measurements illustrated in Figs. 5 and 6. It should be understood that optical parameters of the samples were not known *a priori*, and lifetime determinations were made without a reference luminophore, being based instead on the phase-shift measurements shown. Results of the two-parameter fits of expression (5) to

the phase-shift data are indicated by curves that join the data symbols obtained at the excitation and the emission wavelengths. The optical coefficients extracted for the samples containing the various concentrations of DTTCI or ICG are summarized in Table 1.

Experiments were also conducted determining lifetime based on relative modulation attenuation measurements with detector 48. Fig. 8 provides relative modulation magnitude versus modulation frequency plots of fluorescence, emission, and excitation characterizations for the two DTTCI concentrations as illustrated by different curve-fitted symbols. Fig. 9 provides relative modulation magnitude versus modulation frequency plots of fluorescence, emission, and excitation characterizations for the two ICG concentrations as illustrated by different curve-fitted symbols. Fig. 10 provides the lifetime calculations for the two concentrations of DTTCI and ICG based on the measurements plotted in Figs. 8 and 9.

All publications, patents, and patent applications cited in this specification are herein incorporated by reference as if each individual publication, patent, or patent application were specifically and individually indicated to be incorporated by reference and set forth in its entirety herein. Furthermore, the following are hereby expressly incorporated by reference: U.S. Patent Number 5,865,754 to Sevick-Muraca et al.; U.S. Patent Number 5,818,583 to Sevick-Muraca et al.; pending U.S. Patent Application Serial Number 09/297,895 to Sevick-Muraca et al. filed on 30 June 1999; pending U.S. Patent Application to Sevick-Muraca et al. entitled "Imaging of Light Scattering Tissues with Fluorescent Contrast Agents" filed 6 August 1999 as a national stage application of International Application Number PCT/US98/02354 (U.S. patent application serial number not yet available); and U.S. Provisional Patent Application Serial Number 60/103,609 to Sevick-Muraca et al. filed on 9 October 1998. While the invention has been illustrated and described in detail in the drawings and foregoing description, the same is to be considered as illustrative and not restrictive in character, it being understood that only the preferred embodiment has been shown and described and that all changes, equivalents, and modifications that come within the spirit of the inventions defined by following claims are desired to be protected.

CLAIMS

What is claimed is:

1. A method, comprising:

interrogating a light scattering medium including an amount of a selected luminophore with light at an emission wavelength of the luminophore;

sensing multiply scattered light at the emission light wavelength in response to said interrogating to provide a first optical characterization of the medium;

exposing the medium to light at an excitation wavelength of the luminophore;

detecting a multiply scattered luminescence at the emission wavelength in response to said exposing; and

determining a value corresponding to lifetime of the luminophore from the first optical characterization and the luminescence.

2. The method of claim 1, further comprising illuminating the medium with the excitation light wavelength and sensing multiply scattered light at the excitation wavelength in response to said illuminating to establish a second optical characterization of the medium.

3. The method of claim 1 or 2, wherein the first optical characterization corresponds to photon density of the medium with respect to the emission light wavelength.

4. The method of any of claims 1-3, wherein the emission light wavelength and the excitation light wavelength are modulated at a predetermined frequency and said sensing and said detecting include determining at least one of a phase difference and a modulation attenuation.

5. The method of any of claims 1-4, wherein said exposing is performed with modulated light of the emission light wavelength, said sensing includes detecting the multiply scattered light at each of a number of different frequencies of the modulated light, and the first optical characterization includes determining a number of values by regression with respect to data obtained from said sensing over the different frequencies.

6. The method of any of claim 1-5, wherein the first optical characterization includes an absorption coefficient and an isotropic scattering coefficient of the medium at the emission light wavelength.

7. The method of any of claims 1-6, wherein the light scattering medium is a living tissue and the luminophore is an exogenous probe introduced into the tissue to perform a medical diagnosis.

8. A method, comprising:

exposing a light scattering medium including an amount of a luminophore to a number of different light wavelengths selected relative to the luminophore;

establishing a plurality of optical characteristics of the medium by sensing multiply scattered light at each of the different wavelengths; and

determining a value corresponding to lifetime of the luminophore from the optical characteristics and a multiply scattered emission at a first one of the light wavelengths caused by exposure of the medium to a second one of the light wavelengths.

9. The method of claim 8, where the first one of the light wavelengths corresponds to an emission wavelength of the luminophore and the second one of the light wavelengths corresponds to an excitation wavelength of the luminophore.

10. The method of claim 8 or 9, wherein the optical characteristics correspond to absorption and isotropic scattering coefficients of the medium at each of the different wavelengths.

11. The method of any of claims 8-10, wherein the different wavelengths are each modulated over time and said exposing and said establishing are performed for each of a number of different modulation frequencies.

12. The method of any of claims 8-11, wherein said establishing includes determining at least one of a phase difference and a modulation attenuation.

13. The method of any of claims 8-12, wherein the light scattering medium is a living tissue and the luminophore is an exogenous probe introduced into the tissue to perform a medical diagnosis.

14. A method, comprising:

evaluating a light scattering medium including an amount of a luminophore with a light interrogation system, the system including a first device optically coupled to a first site of the medium and a second device optically coupled to a second site of the medium spaced apart from the first site;

subjecting the medium to a first light wavelength selected relative to the luminophore;

optically coupling the first device to the second site and the second device to the first site;

exposing the medium to the first light wavelength after said optically coupling; and

determining a value corresponding to lifetime of the luminophore from a first light output detected in response said subjecting and a second light output detected in response another of said exposing.

15. The method of claim 14, wherein the first device includes a first light sensor, the second device includes a second light sensor, and the first light wavelength corresponds to at least one of an emission wavelength and an excitation wavelength of the luminophore.

16. The method of claim 14 or 15, wherein the first light output and the second light output are each of the first light wavelength.

17. The method of any of claims 14-16, wherein the first light wavelength corresponds to an emission wavelength of the luminophore; and said subjecting, said optically coupling, and said exposing are performed to provide a light scattering characterization of the medium at the emission wavelength, the first output light and the second output light being of the first light wavelength.

18. The method of any of claims 14-16, wherein the first light wavelength corresponds to an excitation wavelength of the luminophore; and said subjecting, said optically coupling, and said exposing are performed to provide a light scattering characterization of the medium at the excitation wavelength, the first light output and the second light output being of the first light wavelength.

19. The method of claim 18, further comprising repeating said subjecting, said optically coupling, and said exposing with a second light wavelength in place of the first light wavelength, the second light wavelength corresponding to an emission wavelength of the luminophore to provide a light scattering characterization of the medium at the emission wavelength, the first light output and the second light output being of the second light wavelength.

20. The method of claim 18, further comprising repeating said subjecting, said optically coupling, and said exposing; the first light output and the second light output being of a second light wavelength corresponding to an emission wavelength of the luminophore.

21. The method of claim 14, wherein the first light wavelength corresponds to an excitation wavelength of the luminophore and the first light output and the second light output are of a second light wavelength corresponding to an emission wavelength of the luminophore.

22. The method of any of claims 14-21, wherein said subjecting and said exposing are performed with a modulated light source spaced apart from the first site and the second site.

23. The method of any of claims 14-22, wherein the first light output and the second light output correspond to at least one of a phase difference and a modulation attenuation.

24. The method of any of claims 14-23, further comprising selectively filtering light according to wavelength.

25. The method of any of claims 1-24, further comprising introducing the amount of luminophore into the medium.

26. The method of any of claims 1-25, wherein the luminophore is a fluorophore.

27. The method of any of claims 1-26, wherein said determining is performed in accordance with a relationship corresponding to multiple light scattering behavior of the medium.

28. The method of claim 27, wherein the relationship is based on a diffusion equation model of multiply scattered light in the frequency domain.

29. An apparatus including an light source apparatus, light detection instrumentation, and means for performing the method of any of claims 1-28.

30. A system, comprising:

light source instrumentation to selectively illuminate a light scattering medium including a luminophore;

detection instrumentation to detect multiply scattered light output from the medium in response to illumination by said light source instrumentation; and

a processor operatively coupled to said detection instrumentation to determine a first optical characterization of the medium from a first multiply scattered light output of a first illumination light wavelength and a second optical characterization of the medium from a second multiply scattered light output of a second illumination light wavelength different than said first illumination light wavelength, said processor being operable to

calculate a value corresponding to lifetime of the luminophore from the first optical characterization, the second optical characterization, and a multiply scattered emission of the luminophore from the medium in response to excitation.

31. The system of claim 30, further comprising a container to receive the medium, that is optically coupled to said light source instrumentation and said detection instrumentation.

32. The system of claim 30 or 31, wherein said light source instrumentation includes a pair of light sources, a first one of the light sources providing light at the first illumination light wavelength and a second one of the light source providing light at the second illumination light wavelength.

33. The system of any of claims 30-32, wherein said light source instrumentation is operable to provide modulated light of the first illumination light wavelength and the second illumination wavelength.

34. The system of any of claims 30-33, wherein the first illumination light wavelength corresponds to an emission wavelength for the luminophore and the second illumination light wavelength corresponds to an excitation wavelength for the luminophore.

35. The system of any of claims 30-34, wherein said detection instrumentation includes a first light sensor optically coupled to a first position in the medium and a second light sensor optically coupled to second position in the medium spaced apart from the first position, said detection instrumentation being reconfigurable to optically couple said first light sensor to said second position and said second light sensor to said first position.

36. The system of claim 35, further comprising a first optical coupler to transmit light from said first position to said first or second sensor, and a second optical coupler to transmit light from said second site to said first or second sensor.

37. The method of any of claims 30-36, wherein said processor calculates said value in accordance with a relationship corresponding to multiple light scattering behavior of the medium.

38. The method of claim 37, wherein said relationship is based on a diffusion equation model of multiply scattered light in the frequency domain.

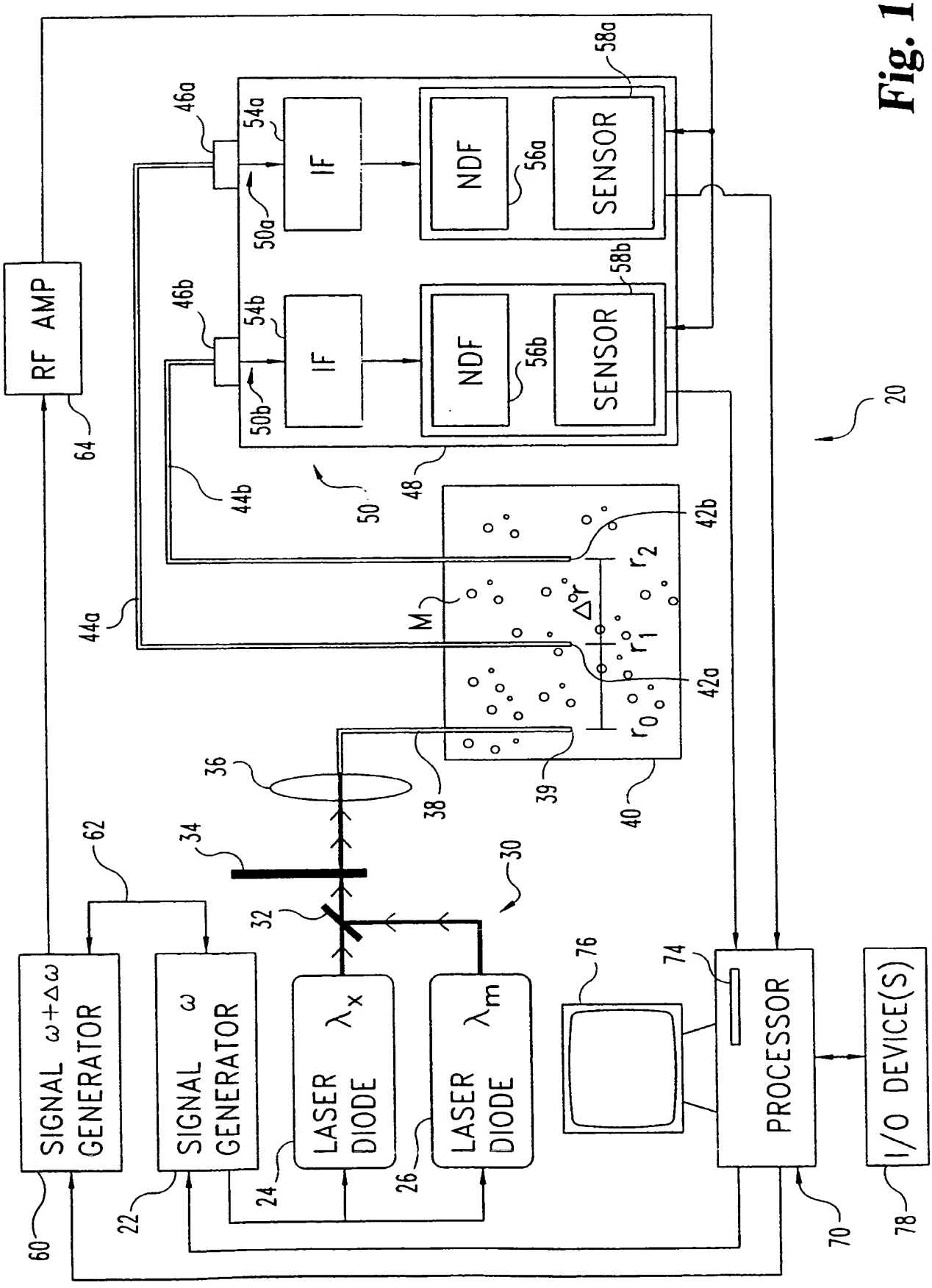
39. The method of claim 30-38 wherein said detection instrumentation provides one or more output signals to said processor corresponding to at least one of a phase difference and a modulation attenuation.

40. A system, comprising:

means for illuminating a light scattering medium including a luminophore; and

means for characterizing light scattering behavior of the medium for an excitation wavelength of the luminophore and an emission wavelength of the luminophore;

means for determining lifetime of the luminophore from said characterizing means and a multiply scattered light emission from the medium at the emission wavelength in response to illumination by light at the excitation wavelength.


RF AMP
64
46a
54a
IF
50a
NDF
56a
SENSOR
58a
46b
54b
IF
50b
NDF
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SENSOR
58b
48
50
20
44a
44b
M
42a
42b
r_0
r_1
r_2
Δr
36
38
39
40
34
30
32
62
SIGNAL $\omega+\Delta\omega$ GENERATOR
SIGNAL ω GENERATOR
LASER DIODE λ_x
LASER DIODE λ_m
22
24
26
60
76
74
PROCESSOR
I/O DEVICE(S)
70
78

Fig. 1

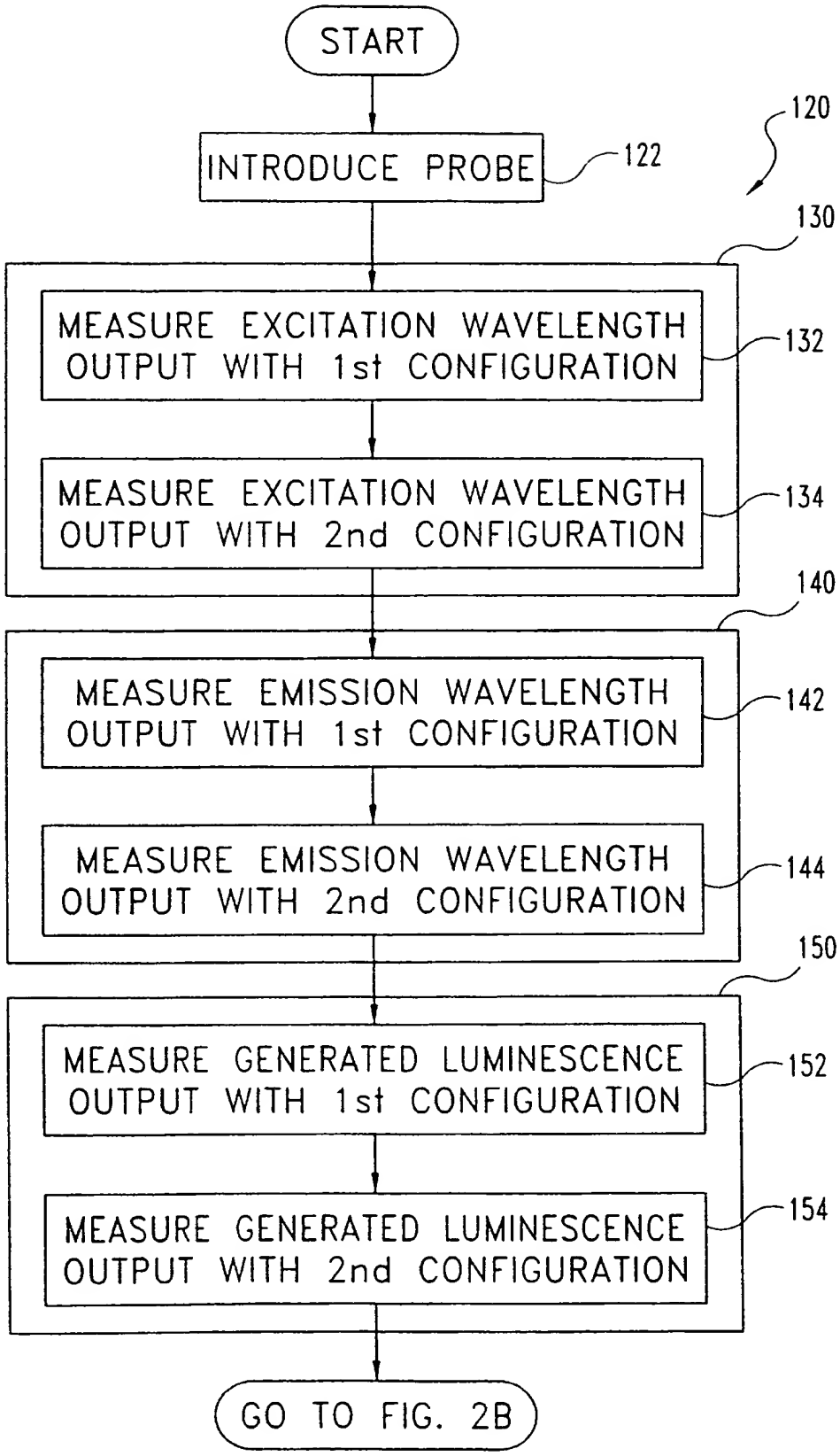
START
INTRODUCE PROBE
122
120
130
MEASURE EXCITATION WAVELENGTH OUTPUT WITH 1st CONFIGURATION
132
MEASURE EXCITATION WAVELENGTH OUTPUT WITH 2nd CONFIGURATION
134
140
MEASURE EMISSION WAVELENGTH OUTPUT WITH 1st CONFIGURATION
142
MEASURE EMISSION WAVELENGTH OUTPUT WITH 2nd CONFIGURATION
144
150
MEASURE GENERATED LUMINESCENCE OUTPUT WITH 1st CONFIGURATION
152
MEASURE GENERATED LUMINESCENCE OUTPUT WITH 2nd CONFIGURATION
154
GO TO FIG. 2B

Fig. 2A

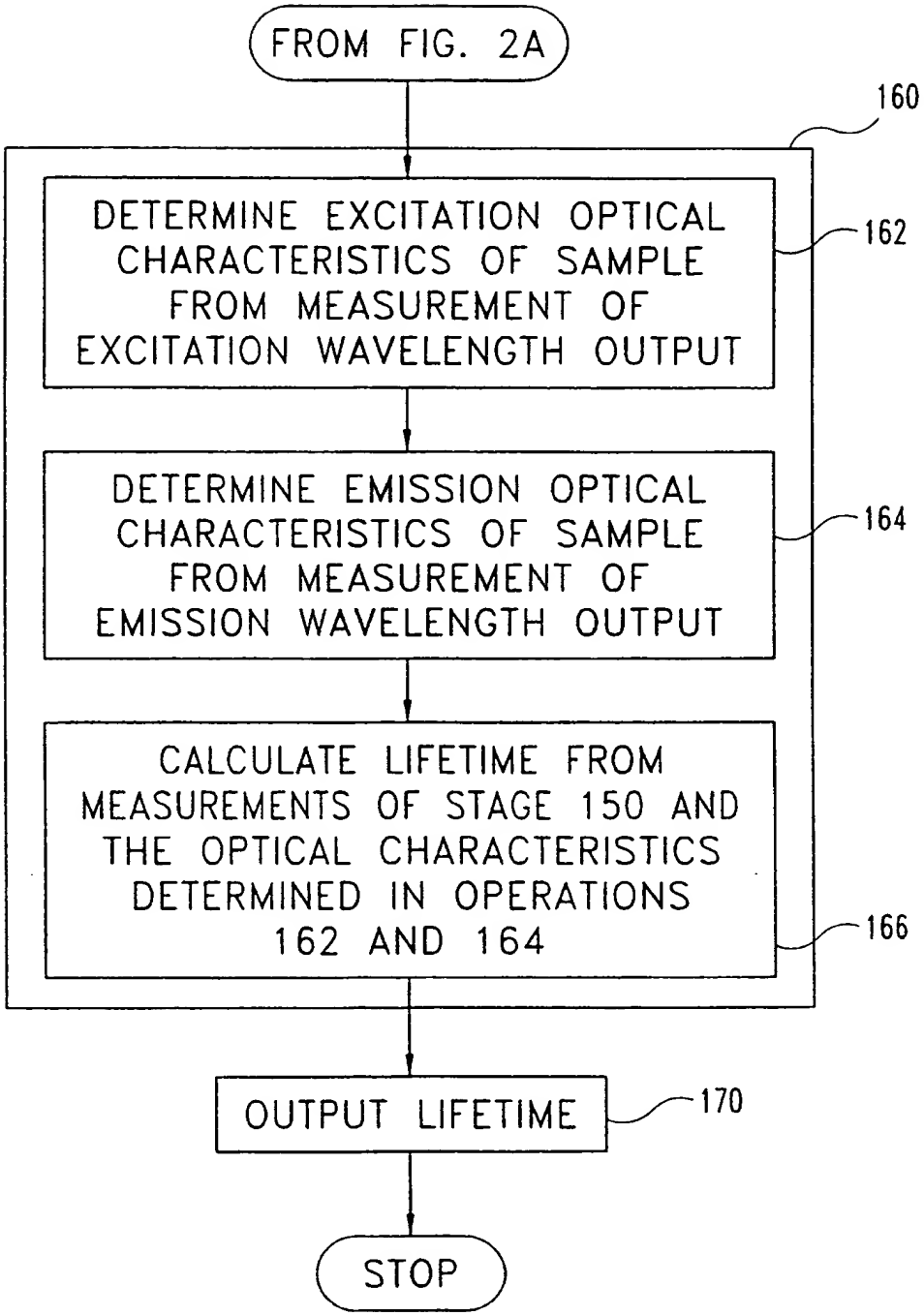
FROM FIG. 2A
160
DETERMINE EXCITATION OPTICAL CHARACTERISTICS OF SAMPLE FROM MEASUREMENT OF EXCITATION WAVELENGTH OUTPUT
162
DETERMINE EMISSION OPTICAL CHARACTERISTICS OF SAMPLE FROM MEASUREMENT OF EMISSION WAVELENGTH OUTPUT
164
CALCULATE LIFETIME FROM MEASUREMENTS OF STAGE 150 AND THE OPTICAL CHARACTERISTICS DETERMINED IN OPERATIONS 162 AND 164
166
OUTPUT LIFETIME
170
STOP

Fig. 2B

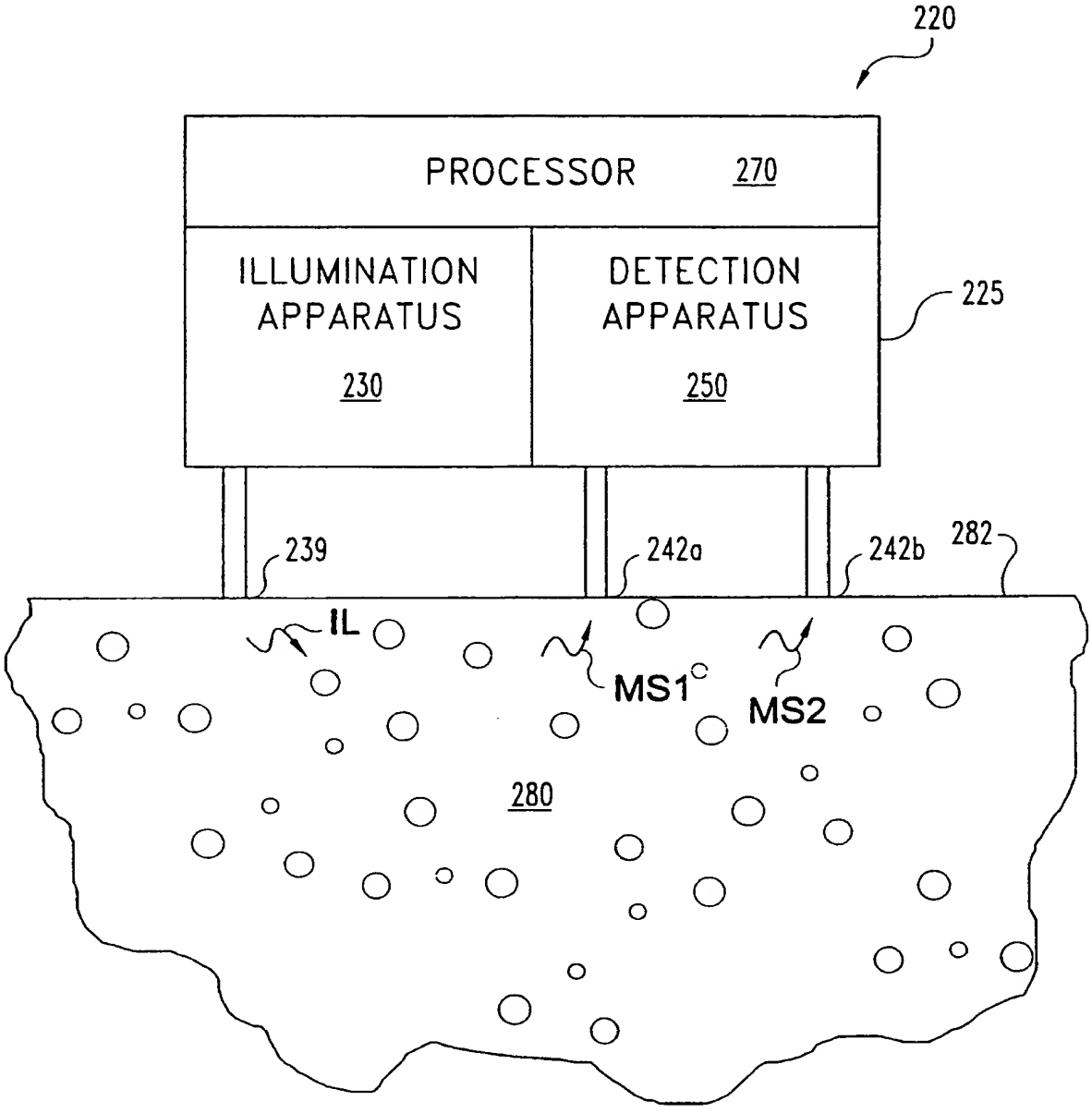
220
PROCESSOR 270
ILLUMINATION APPARATUS 230
DETECTION APPARATUS 250
225
239
242a
242b
282
IL
MS1
MS2
280

Fig. 3

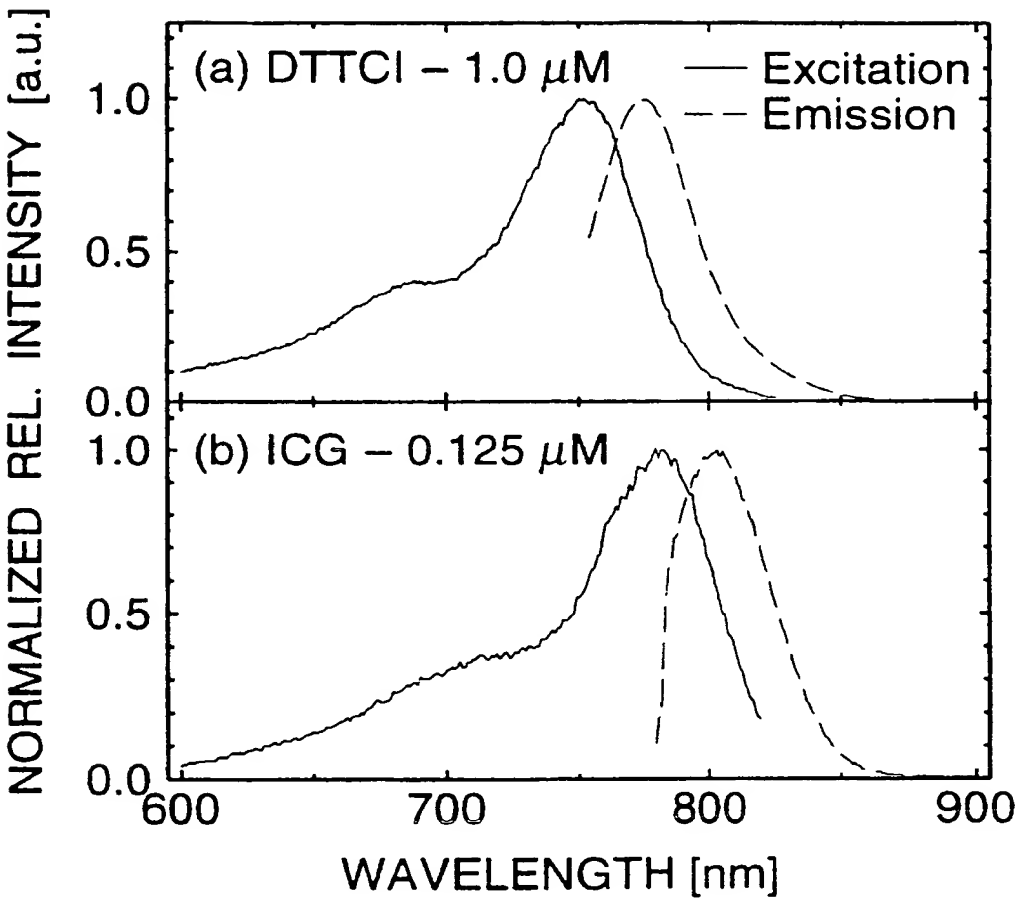
(a) DTTCI – 1.0 μM
Excitation
Emission
(b) ICG – 0.125 μM
NORMALIZED REL. INTENSITY [a.u.]
1.0
0.5
0.0
600
700
800
900
WAVELENGTH [nm]

Fig. 4

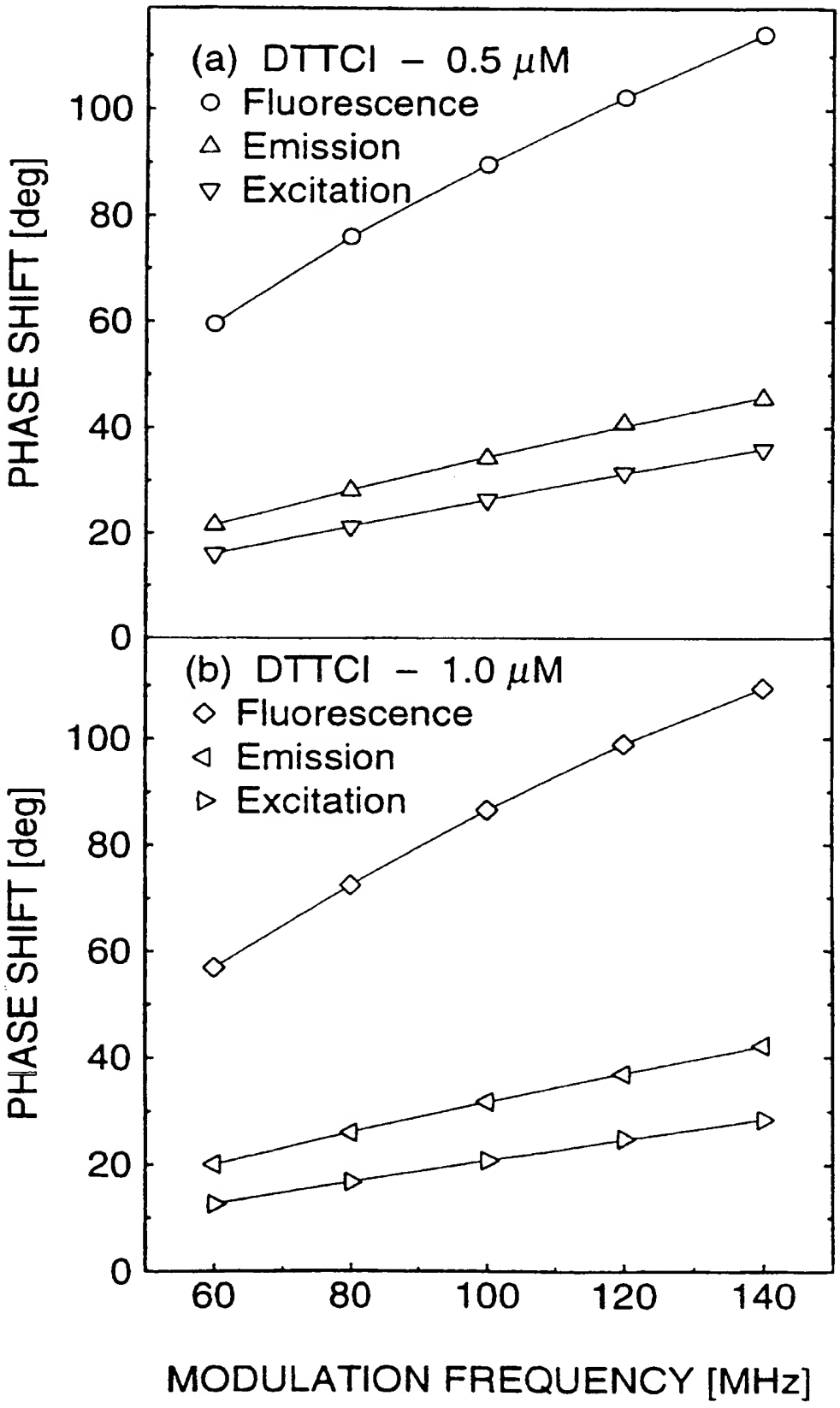
(a) DTTCI – 0.5 μM
Fluorescence
Emission
Excitation
(b) DTTCI – 1.0 μM
Fluorescence
Emission
Excitation
PHASE SHIFT [deg]
PHASE SHIFT [deg]
0
20
40
60
80
100
60
80
100
120
140
MODULATION FREQUENCY [MHz]

Fig. 5

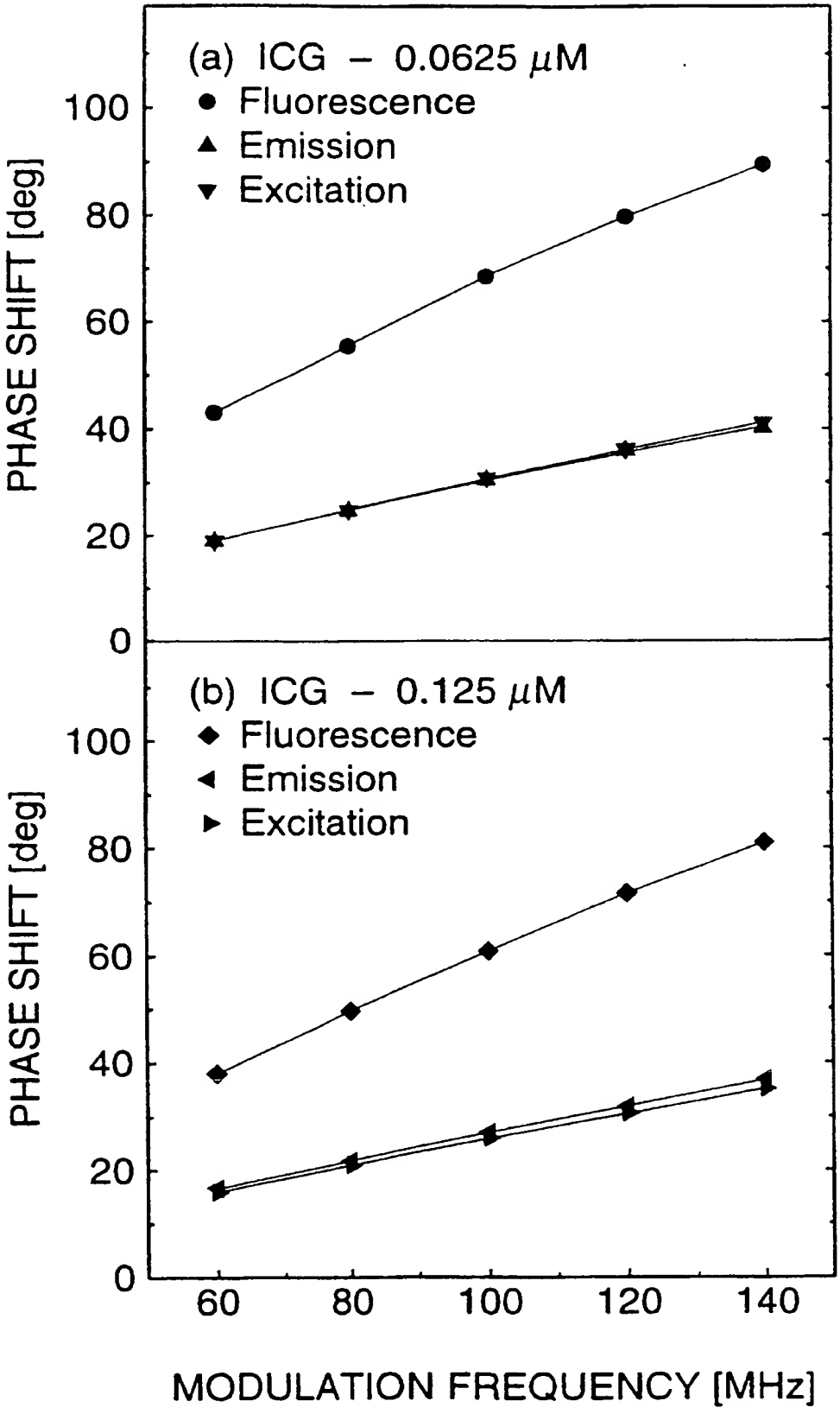
(a) ICG – 0.0625 μM
Fluorescence
Emission
Excitation
PHASE SHIFT [deg]
0
20
40
60
80
100
(b) ICG – 0.125 μM
Fluorescence
Emission
Excitation
PHASE SHIFT [deg]
0
20
40
60
80
100
60
80
100
120
140
MODULATION FREQUENCY [MHz]

Fig. 6

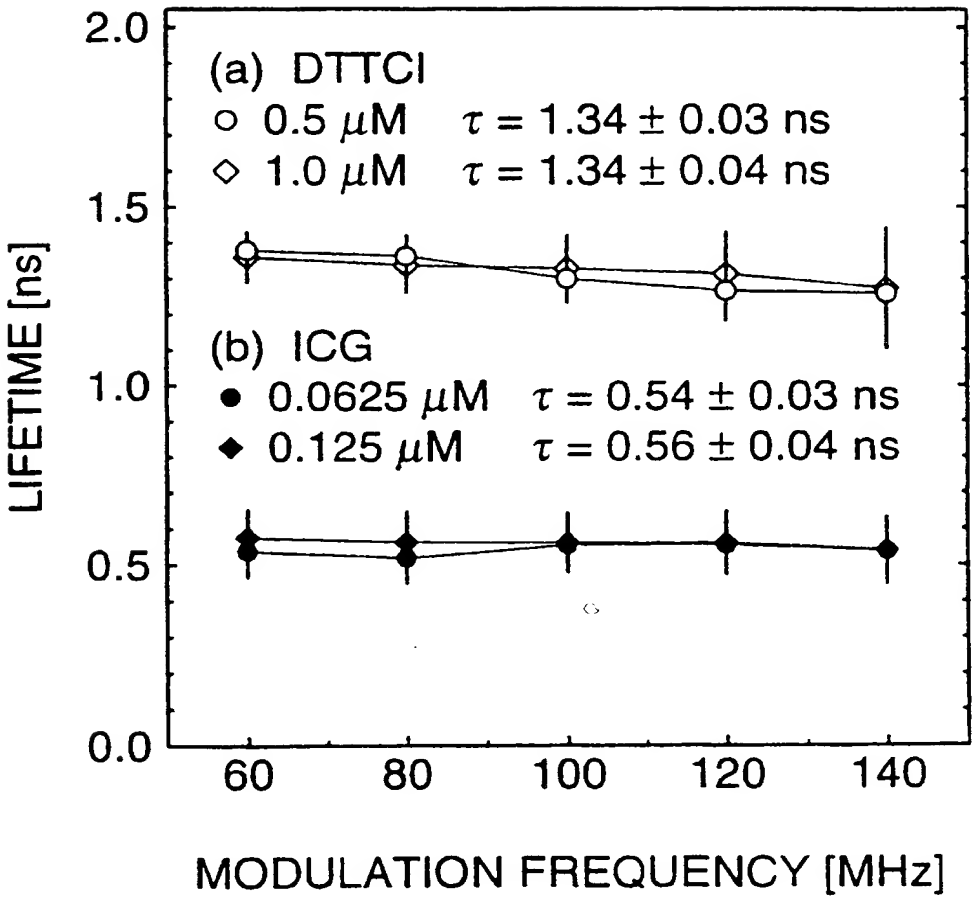
2.0
1.5
1.0
0.5
0.0
LIFETIME [ns]
(a) DTTCI
○ 0.5 μM τ = 1.34 ± 0.03 ns
◇ 1.0 μM τ = 1.34 ± 0.04 ns
(b) ICG
● 0.0625 μM τ = 0.54 ± 0.03 ns
◆ 0.125 μM τ = 0.56 ± 0.04 ns
60
80
100
120
140
MODULATION FREQUENCY [MHz]

Fig. 7

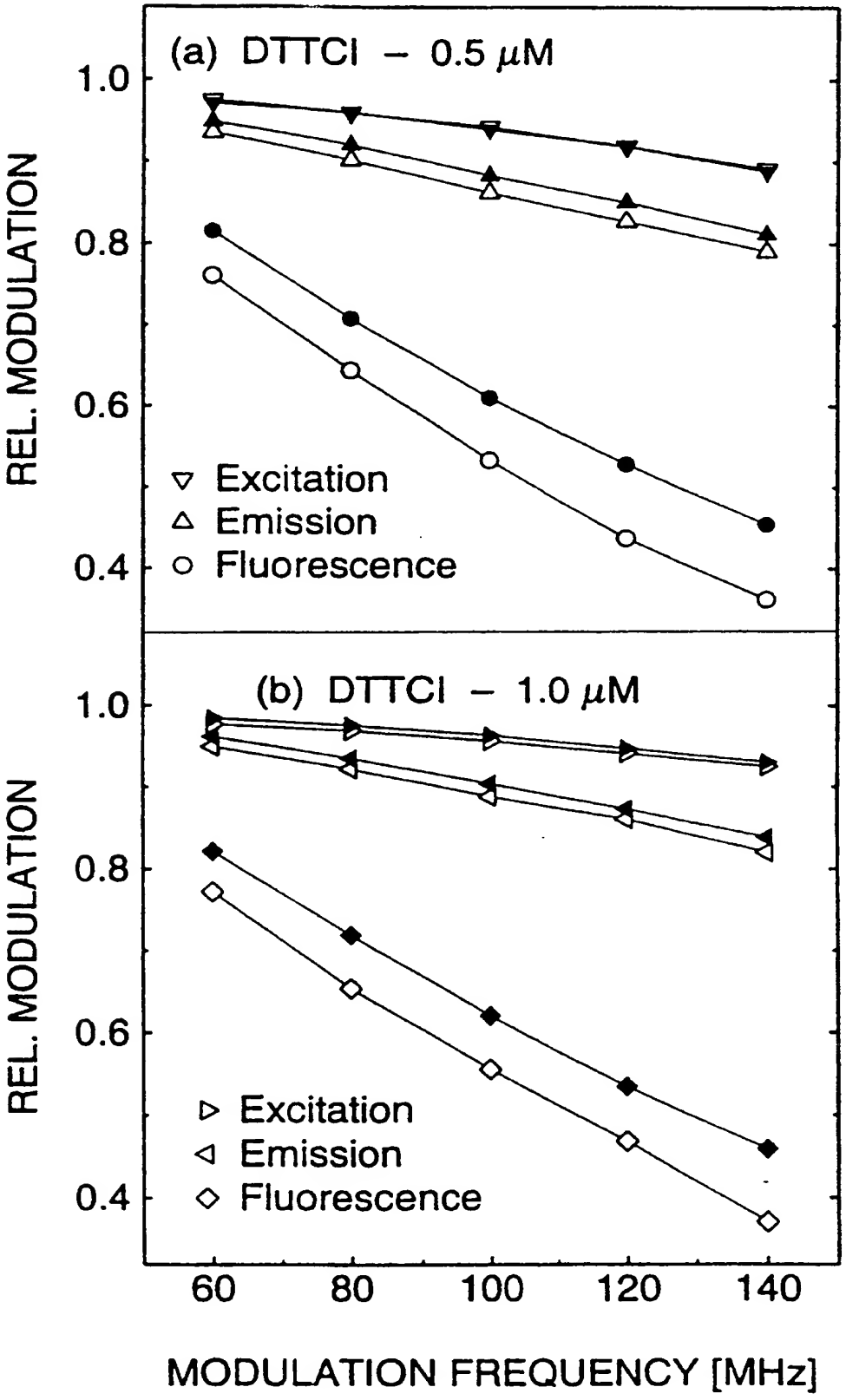
(a) DTTCI – 0.5 μM
1.0
0.8
0.6
0.4
REL. MODULATION
Excitation
Emission
Fluorescence
(b) DTTCI – 1.0 μM
1.0
0.8
0.6
0.4
REL. MODULATION
Excitation
Emission
Fluorescence
60
80
100
120
140
MODULATION FREQUENCY [MHz]

Fig. 8

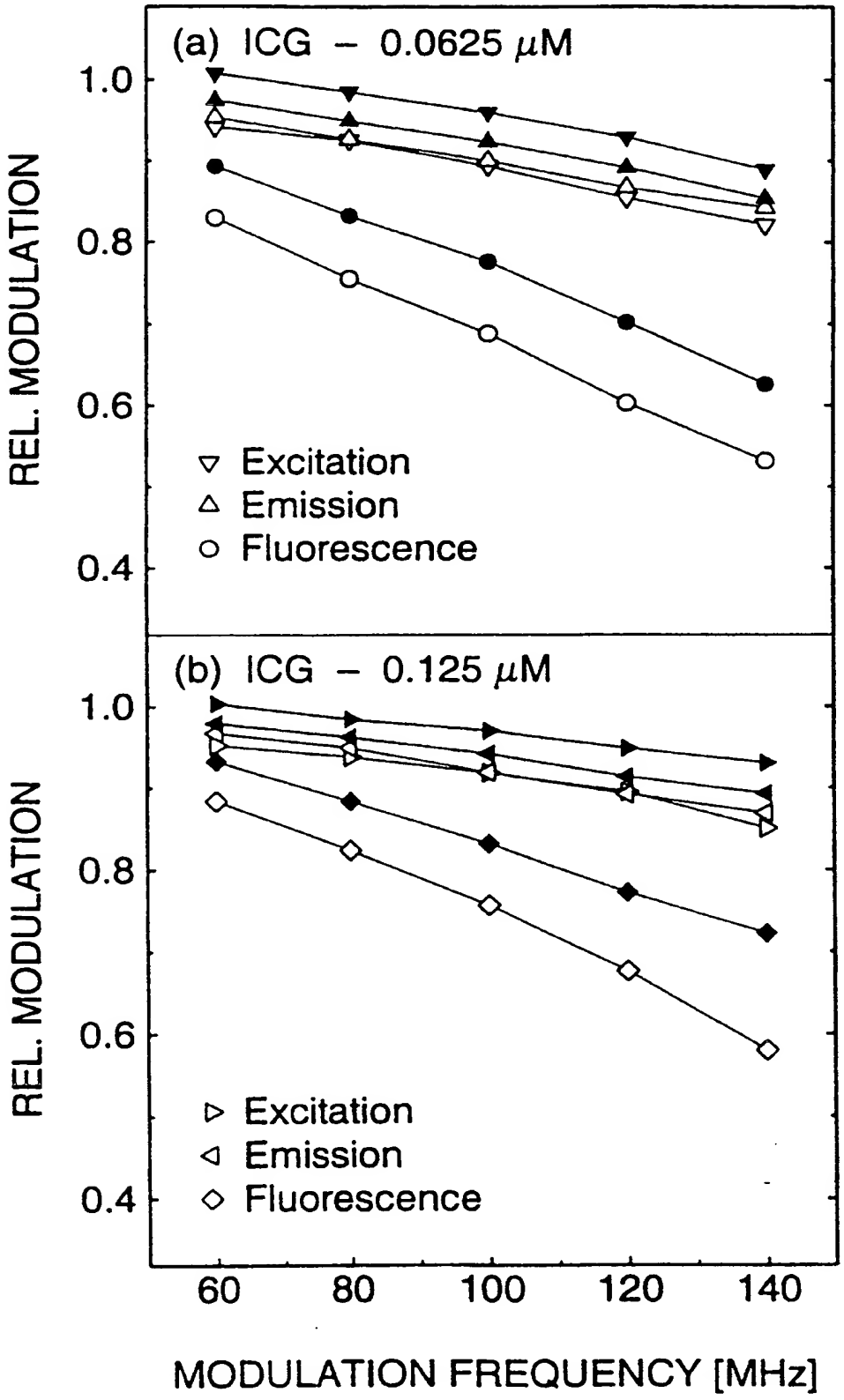
(a) ICG – 0.0625 μM
1.0
0.8
0.6
0.4
REL. MODULATION
Excitation
Emission
Fluorescence
(b) ICG – 0.125 μM
1.0
0.8
0.6
0.4
REL. MODULATION
Excitation
Emission
Fluorescence
60
80
100
120
140
MODULATION FREQUENCY [MHz]

Fig. 9

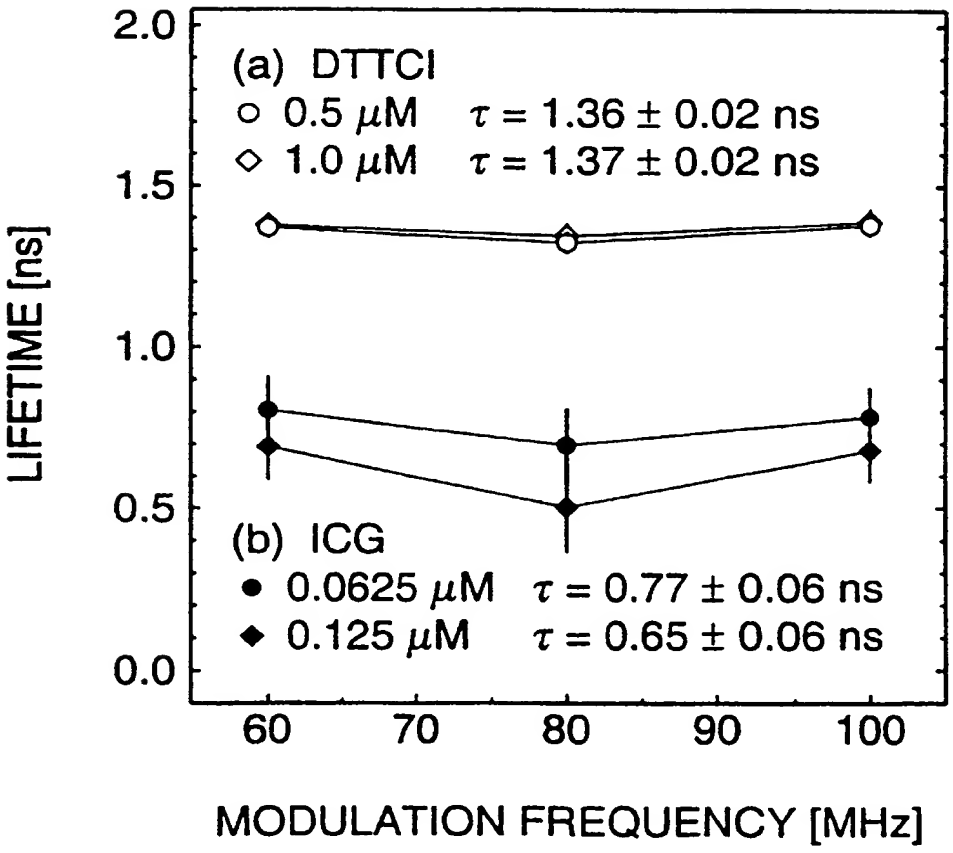
2.0
1.5
1.0
0.5
0.0
LIFETIME [ns]
(a) DTTCI
○ 0.5 μM τ = 1.36 ± 0.02 ns
◇ 1.0 μM τ = 1.37 ± 0.02 ns
(b) ICG
● 0.0625 μM τ = 0.77 ± 0.06 ns
◆ 0.125 μM τ = 0.65 ± 0.06 ns
60
70
80
90
100
MODULATION FREQUENCY [MHz]

Fig. 10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/23709

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :G01N 21/00
US CL :356/440

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 356/440, 317, 337, 338; 436/172

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

US PTO APS EAST search terms: luminophore, luminescence, lifetime, multiply scattered, emission wavelength, excitation wavelength

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,818,583 A [SEVICK-MURACA ET AL] 06 October 1998 (06/10/98), see entire document, especially figure 1 and columns 5-7.	1-3, 8-10, 14-16, 21, 30-32, and 40
Y	US 5,624,847 A [LAKOWICZ ET AL] 29 April 1997 (29/04/97), see entire document.	1-3, 8-10, 14-16, 21, 30-32 and 40
Y	US 5,736,410 A [ZARLING ET AL] 07 April 1998 (07/04/98), see entire document.	1-3, 8-10, 14-16, 21, 30-32, and 40
A	US 5,190,729 A [HAUENSTEIN ET AL] 02 March 1993 (02/03/93), see entire document.	1-3, 8-10, 14-16, 21, 30-32, and 40

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
16 DECEMBER 1999	12 JAN 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer AMANDA MERLINO Telephone No. (703) 305-3488

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/23709

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-7, 11-13, 17-20, 22-29, and 33-39
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.